International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Determination of Hydroquinone in Some Pharmaceutical and Cosmetic Preparations by Spectrophotometric Method

Hawaa S. Elferjani[1], Najw H. S. Ahmida[2], Aziza Ahmida[3]

[1, 2, 3]Department of Chemistry, Faculty of Science, University of Benghazi, Libya 2017

Abstract: ***In This study thirteen skin lighting creams are available in Libyan markets in Benghazi and Pharmaceutical Preparations were used to determine the content of hydroquinone by a rapid and simple UV spectrophotometric procedures. The labels on the packages noticeably did not show the content of hydroquinone. All samples were analysed by a UV spectrophotometer, Beer's law was obeyed in the range of 10- 40 µg/ml at 290nm using 0.05M H_2SO_4 as solvent with linear regression coefficient of 0.9994. The results showed the concentration of hydroquinone in all cosmetic samples ranged from 0.008% to 0.210 %. The effect of time has been studied.***

Keywords: hydroquinone, spectrophotometry, pharmaceutical and cosmetic preparations

1. Introduction

Hydroquinone is an aromatic organic compound of the phenol, a derivative of benzene having the chemical formula $C_6H_4(OH)_2$, figure].

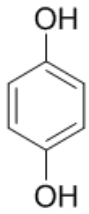

Figure 1: Chemical structure of hydroquinone

It is occurs in nature in many different forms. It appears in the leaves and bark of certain berries like blueberries and cranberries. If tea is made from these leaves, it will then appear in the tea. It is in certain coffee beans and is therefore in certain coffees. It can also be found in various fruits and vegetables, and wheat. It can therefore also be found in wines and beers. Industrially, it is produced in several countries. In 1979, the total world capacity for production exceeded 40000 tonnes, while in 1992 it was approximately 35000 tonnes. It is extensively used as a reducing agent, as a photographic developer, as an antioxidant or stabilizer for certain materials that polymerize in the presence of free radicals, and as a chemical intermediate for the production of antioxidants, agrochemicals and polymers. Hydroquinone is also used in cosmetics and medical preparations. Hydroquinone is used as a topical application in skin whitening to reduce the colour of skin. Hyperpigmentation skin disorder is treated with hydroquinone products, this substance inhibits the tyrosinase enzyme which is responsible for the first reaction of melanin formation [1] Subsequently melanin decreases and skin becomes depigmented . It is known that hydroquinone is the most conventional skin whitening agent, however ,Clinical preparation containing 2-4% hydroquinone are prescribed for the treatment of hyperpigmentation such as melisma, freckles and senile lentigines as well as chloasma. In addition, numerous studies revealed that, Hydroquinone has numerous unfavourable effects with long term applications including irritative dermatitis melanocyte destruction, contact dermatitis and ochronosis [2,3]. its use has been recommended to ban in cosmetics[4,5]; it is still being used in developing countries in skin lightening cosmetics. numerous studies point to hydroquinone as a likely carcinogen. Numerous studies show a high occurrence of tumors in rats who had been subjected to doses of hydroquinone, including thyroid follicular cell hyperplasias, anisokaryosis, mononuclear cell leukemia, hepatocellular adenomas, renal tubule cell adenomas. Hydroquinone toxicity can lead to severe side effects such as kidney and liver malfunction, blood poisoning, nausea, abdominal pains, convulsion and even coma. Animal test on rats, mice and rabbits showed that hydroquinone can cause acute toxicity, all of these potentially lethal results are possibilities with the use of too much hydroquinone. As a result of these tests the European Union has banned its use. However, Terer *et. al*. [6] reported the content of hydroquinone in Body lotions and creams sold in Retail outlet in Barton, Kenya. In this study twenty four body lotions and body creams were randomly sampled from the retail outlets within Baraton, the lables on the packages noticeably did not indicate the presence of hydroquinone. The level of hydroquinone for all samples creams was below 2% which is upper limit for cosmetic creams. Ansah and co-workers [7] studied the content of hydroquinone of skin toning creams and cosmetic soaps, in this study sixty –two skin lighting creams and soaps were analysed to detect the hydroquinone levels by high performance liquid chromatography. The mean concentration of total hydroquinone was 0.234± 0.385 and 0.035±0.021% in skin toning creams and cosmetic soaps. All the creams and soaps analysed had hydroquinone levels below the US food and Drug administration's accepted limited 1µg/g and 2% respectively. hydroqunione is carcinogenic it has been banned in some countries because of fears of a cancer risk [8,9]. Hydroquinone has been used for decades as a skin lightening agent. Metabolites of hydroquinone formed in the liver, e.g., p-benzoquinone and glutathione conjugates of hydroquinone are the main cancer-causing agents. In the bone marrow, hydroquinone is

oxidized into p-benzoquinone because of the high myeloperoxidase activity. Topically applied hydroquinone-containing creams may give rise to accumulation of p-benzoquinone and glutathione conjugates of hydroquinone. These compounds are also responsible for the DNA damage and mutations. They also have the capability to disrupt protective mechanisms, whereby they facilitate further development of cancer.

In the bone marrow, long-term effects such as aplastic anemia and acute myeloid leukemia may occur. Most of the evidence stems from research on benzene toxicity, which appears to arise via its metabolite hydroquinone. There is no report yet demonstrating carcinogenesis or other ill-effects resulting from the application of hydroquinone-containing creams. The fact that many countries around the world have banned Hydroquinone is no coincidence - hydroquinone based products have caused disfigurement and permanent scarring to hundreds of thousands of faces around the world. Dermatologists say prolonged use of Hydroquinone products destroys the skin's protective outer layer and may cause temporary or permanent discoloration of the skin. Ultimately, it can damage the nerves or even lead to kidney failure or skin cancer [10]. Neurological effects of hydroquinone include; headache, dizziness, tinnitus, delirium, muscle twitching, tremor, nausea, vomiting, and the production of green to brown-green urine may occur[11]

Chemical and reagents
All reagents were of analytical reagent grade (BDH Chemicals Ltd, Poole, England))

Reagents required
Sulphuric acid (0.05M),

A Standard solution of Hydroquinone
(1.00g, M.W.= 110.112 g/mol) standard hydroquinone was dissolved in 1000 ml of 0.05 M sulphuric acid to make 1000 ppm

Sampling
In this study thirteen different brands of cosmetic cream randomly selected from Benghazi market, and three Pharmaceutical Preparations we are using the method proposed by (Oyedeji *et al*., 2009) to analyze the presence and exact concentration of Hydroquinone. For the samples, about accurately 1g of each sample was dissolved in 20 cm^3 of 0.05M Sulphuric acid in a water bath. This solution was then transferred into 25cm^3 standard volumetric flask and made to volume with the 0.05M sulphuric acid. The solution was then filtered with a filter paper then discarded the first 5 cm^3 . The filter paper was then rinsed with additional 5 cm^3 of sulphuric acid to remove any retained sample. The concentration of hydroquinone was determined using a UV spectrophotometer at a wavelength of 290 nm. using quartz cuvette.

Estimation of λmax (maximum absorption of hydroquinone)
To determine the maximum absorption, standard solutions of hydroquinone in concentration of 40 ppm were prepared. Scanning of the hydroquinone in a wavelength range from 250 nm to 320 nm showed a maximum absorbance (λmax) at 290 nm as shown in table (1) and figure(2)

Table 1: Maximum absorbance wavelength (λ_{max}, nm)

λ(nm)	A
250	0.087
260	0.134
270	0.321
280	0.645
290	0.974
295	0.792
300	0.612
310	0.065
320	0.019

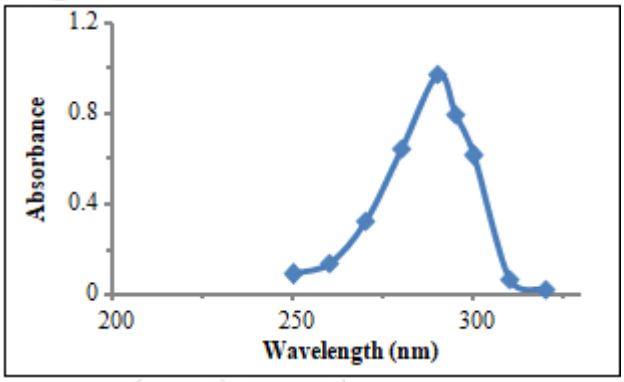

Figure 2: The absorption spectrum of hydroquinone solution (40µg/ml) in 0.05 M H_2SO_4

The most important objective gets from fig.2 is the detection of maximum wavelength (λ_{max}) for hydroquinone Which was 290 nm. This λ_{max} is used for further quantitative spectrophotometric measurements. This λ_{max} for hydroquinone was similar to the wavelength used for other spectrophotometric measurements by different authors [12-15]

Calibration curve
After determination of the maximum absorption of hydroquinone (290 nm) using spectrophotometer, The absorbance was then taken at a wavelength of 290 nm and traced on the calibration curve to give the concentration of hydroquinone in each samples. The calibration curve was obtained from Hydroquinone standard by serial dilutions of concentrations 10,15,20,25,30,35 and 40 ppm. Under the optimum experimental conditions, a good linear correlation was obtained between the absorbance and hydroquinone concentration in the range from 10 to 40 µg/ml, fig. 3.

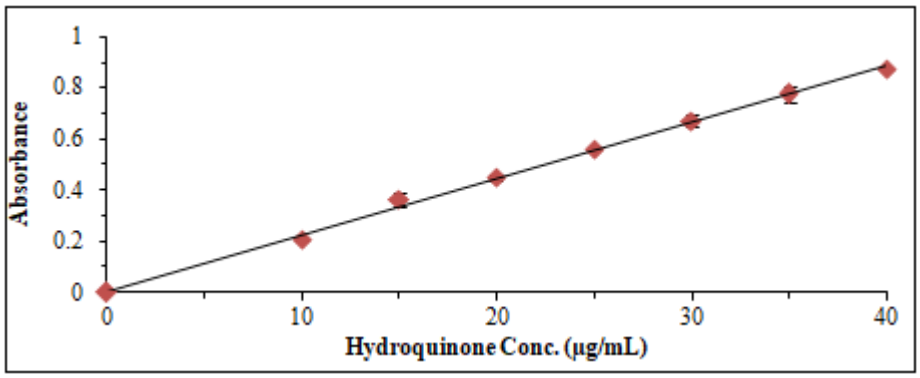

Figure 3: The calibration curve of hydroquinone (10-40 µg/ml) in 0.05 M H_2SO_4 solution at 290 nm. The linear regression equation is $A = 0.022C + 0.007$ ($R^2 = 0.997$)

From table 2; the parameters of the hydroquinone concentration-absorbance straight line were calculated by the least-squares method. The regression equation of the calibration line has the form:

$$A = 0.022C + 0.007$$
$$(R^2 = 0.997).$$

where *C* is the concentration of hydroquinone (µg/mL) and *A* is the absorbance. The correlation coefficient (R^2) is 0.997. Statistical evaluation of the regression line using standard deviation about the regression (S_r), the standard deviation of intercept (S_a) and standard deviation of the slop (S_b) gave the following values: 1.60×10^{-2}, 4.53×10^{-4}, and 1.14×10^{-2} respectively. These small values point out low scattering of the point around the calibration curve and to the high precision of the method [16,17]. The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be detected. The LOD was found to be 2.358µg/mL, according to the 3s/m definition [43], where ***s*** is the standard deviation (n=6) of the signal from 30µg/ml hydroquinone aliquots, and ***m*** is the slop of the calibration graph. The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured with acceptable accuracy and precision and was found to be 7.858 µg/mL.

Table 2: Analytical data and the optical characteristics for the determination of Hydroquinone

Parameter	*Analysis of Hydroquinone*
λ_{max}	290 nm
Linear range	10-40 µg/mL
Linear regression equation (A=mC+a)	
Intercept (a)	0.007
Slop (b)	0.022
Correlation coefficient (R^2)	0.997
Limit of detection	2.358 µg/mL
Limit of quantification	7.858 µg/mL

Molar Absorptivity

Whereas molar absorptivity it is very important characteristic to see how accurate and sensitive method. Molar absorptivity was measured at different concentration at 290 nm It was average (2444.974 Mol^{-1} Cm^{-1} L) as shown in table 3

Table 3: Molar absorptivity of hydroquinone at different concentration at290nm

$C_{\mu g/ml}$	C g/L	C Mol/L	A	ϵ Mol^{-1} Cm^{-1} L
10	0.01	9.09091E-05	0.208	2288
15	0.015	0.000136364	0.339	2486
20	0.02	0.000181818	0.447	2458.5
25	0.025	0.000227273	0.555	2442
30	0.03	0.000272727	0.678	2486
35	0.035	0.000318182	0.788	2476.571
40	0.04	0.000363636	0.901	2477.75
Mean				2444.974

Stability Study

Hydroquinone is an organic compound belongs to phenolic group. It is alike the most compounds of this group, easily undergoes oxidative degradation particularly; in presence of metallic ions, high concentration of oxygen; high pH and exposure to light [**18**].

Therefore, the stability of hydroquinone solution is a critical parameter. In this study, the stability of hydroquinone standard solution is evaluated by measuring the absorbance of different concentrations of hydroquinone solutions in sulphuric acid at 290 nm within 21 days. However, the hydroquinone standards solution were kept in a dark at room temperature. The time (days) versus the absorbance corresponding to each concentration of hydroquinone was plotted, as illustrated in fig 4. This figure shows that the absorption response, corresponding to each concentration, remained constant within three weeks. Fig 4, also indicated that stability of hydroquinone standard solution could be even longer.

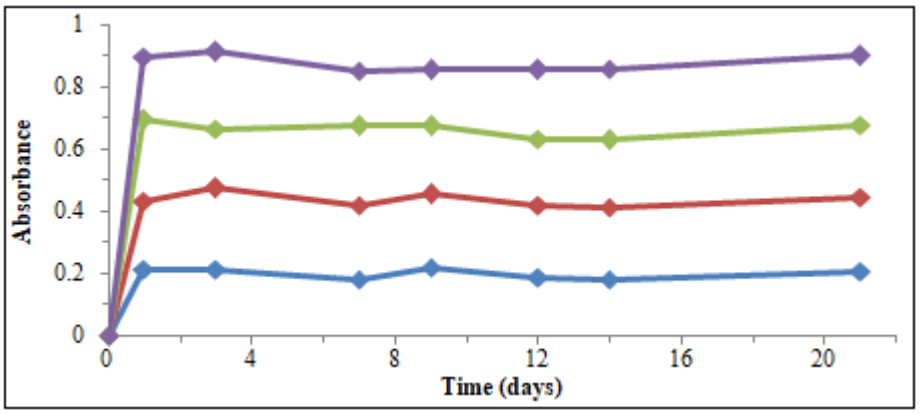

Figure 4: Absorbance versus time graphs for different concentrations of hydroquinone,10 µg/mL (♦), 20 µg/mL (♦), 30 µg/mL (♦) and 40 µg/mL (♦).

Practical Application

After estimation (identifying the maximum wavelength for absorption measurement and obtained the best working range for hydroquinone in standard solution, the method was applied for quantitative determination of hydroquinone in some pharmaceutical creams and skin lighting cosmetics. The result obtained for selected measuring concentration of hydroquinone is given are given in table 4 and 5 respectively and Figures 5 and 6.

Analysis of Pharmaceutical Preparations:

The spectrophotometric method has been applied to determine the concentration of hydroquinone in some pharmaceutical creams, that known in the market. In this project, the commercially pharmaceutical Creams of hydroquinone (Kinon®, Eldoquin® and Alphahydroquinon® creams) were determined directly by measuring the absorbance of the sample solution after the required digestion, filtration and dilution steps. In these studies, the level of hydroquinone is detected at 2.65%, 2.89% and 1.92% for Kinon, Eldoquin and Alpha hydroquinone respectively (table 4). The hydroquinone concentration in all selected pharmaceutical creams were within the acceptance level permitted by WHO.

Table 4: Application of the spectrophotometric method for determination of hydroquinone in pharmaceutical creams

Preparation	*Found (µg/mL)*[a b]	*Found (g%)*[c]
KINON® WHITENING CREAM (hydroquinone 2%), *Alkhuraiji Factory Industrial Permission Riyadh-Kingdom of Saudi Arabia*	33.48±2.23	2.65±0.20
ELDOQUIN® 2% CREAM (hydroquinone 2%), ICN Pharmaceuticals, Inc, Costa Mesa, CA, USA	36.27±3.31	2.89±0.27
Hydroquinone Alpha 2 Cream, Turkey	24.18±0	1.92±0.0

[a] *Mean of 6 values.*
[b] *amount calculated using calibration curve.*
[c] *g%= [Amount found (µg/mL) / sample weigth×25]*

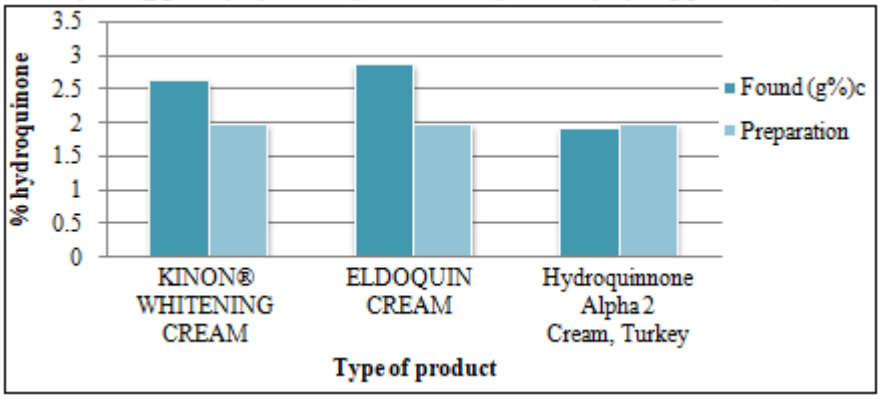

Figure 5: Illustrate Amount hydroquinone for calculated and labelled in g%

Analysis of cosmetics

Various skin whiting creams were collected from local markets. The concentrations of hydroquinone in the selected cosmetic samples were ranged from 0.0077% to 0.21 % as shown in table 5 and figure 6. The level of hydroquinone in the samples was less than 2%, which is level permitted by WHO [15].

Table 5: Analysis of Hydroquinone in Some Skin whitening Cosmetics by the spectrophotometric method

Preparation	Found (µg/mL)	Found (g%)
Spotless Face Cream, Eva cosmetic Co. Egypt.	29.38	0.073
Fair& Lovely, Hindustan Unilever Limited brand, India	31.87	0.080
Flormar BB made in Turkey	6.84	0.017
Nivea For men made in Germany	21.33	0.053
Olay made in U.S.A	44.44	0.210
Nivea for Woman Made in Germany	3.07	0.008
Garnier made in Egypt	27.20	0.140
Essence BB made in Italy	32.62	0.081
NeoRetin Made in Spain	25.156	0.063
Fair and lovely herbel balance Made in India	27.956	0.069
Garneir BB Made in France	35.689	0.089
Hyalufactor made in Switzerland	7.333	0.018
Shirley made in Tawan	14.622	0.037

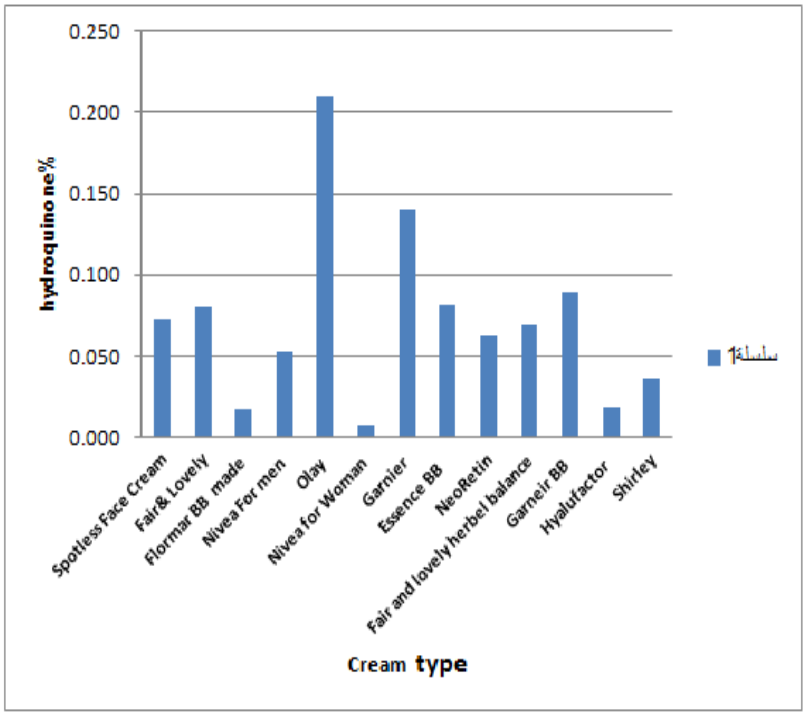

Figure 6: Illustrate level of hydroquinone in cosmetic samples

When the comparison is made with previous studies found that the concentration range of hydroquinone. The level of hydroquinone was below 2% for seven of the creams, between 2 - 5% for two and above 5% for one. (The upper limit for cosmetic creams is 2 and 5% for therapeutic use) [19]. However, Terer *et. al*. reported the content of hydroquinone in Body lotions and creams sold in Retail outlet in Barton, Kenya. In this study twenty four body lotions and body creams were randomly sampled from the retail outlets within Baraton, the labels on the packages noticeably did not indicate the presence of hydroquinone. The level of hydroquinone for all samples creams was below 2% which is upper limit for cosmetic creams. (0.00009 - 0.03475) [20]

2. Conclusion

In our group Thirteen Skin whiting creams in Libyan market and three Pharmaceutical Preparations, have been analysed to determine the content of hydroquinone on it by using spectrophotometric method. The suitable wave length is 290 nm. Beer's law was obeyed in the range of 10- 40 µg/ml at 290nm with linear regression coefficient of 0.9994. The results showed the concentration of hydroquinone in all cosmetic samples ranged from 0.008% to 0.210 % . From the investigation, it is evident that most of creams contain small amounts of hydroquinone that are within the acceptable range. Therefore these levels may not cause permanent skin damage, disfigurement nor are carcinogenic.

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